Protein adsorption to lipid membranes through metal ion chelation studied by X-ray and neutron reflectivity and GIXD

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Outline

I. Introduction - motivation, description of lipid/protein system

II. Results

a. Protein conformation / orientation (NR, XR)

-final state

-evolution of layer structure with time

b. Evidence for two stages (GIXD, NR, XR)

-stage 1: reversible

-stage 2: irreversible

III. Summary

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Introduction

Motivation for studying interaction of proteins with lipid membranes
(membrane-associated, not integral membrane proteins)

a). biochemical processes: protein binding and conformational changes regulate ions channels, play important roles in cellular communication, immune response, etc.

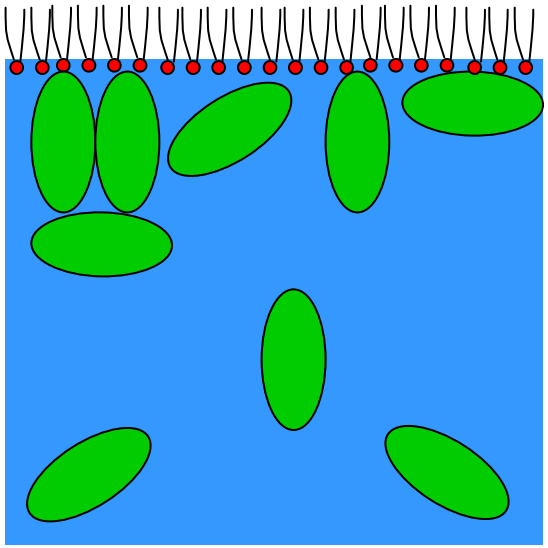
b. nanoscience: control/direct the formation and growth of supramolecular structures (motor protein highways, protein complexes)

c). mechanisms of toxin assault on cell membranes

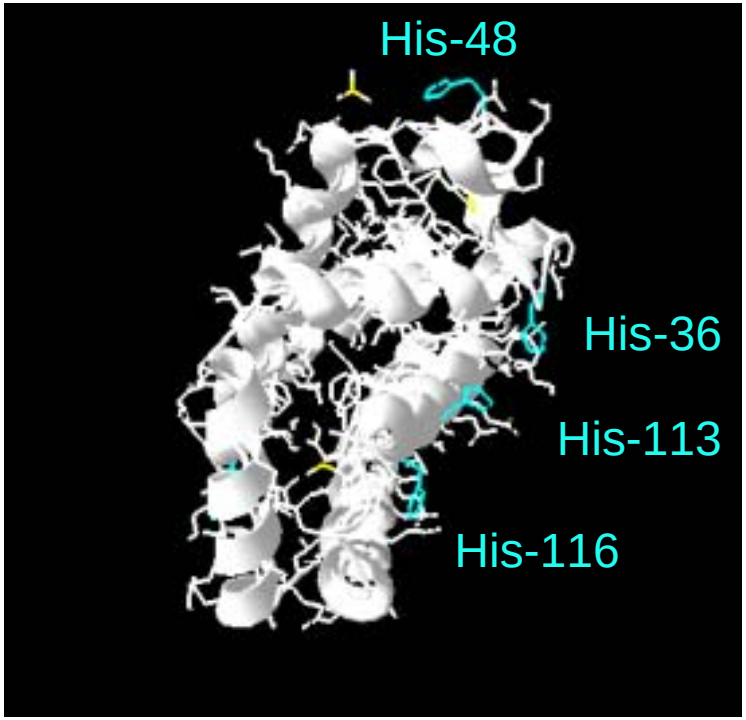
d). biosensors - binding modes determine chemical signals, dictate sensor response, orientation of antibodies

Metal-ion coordination with histidines

Langmuir monolayers of metal-chelating lipids



myoglobin (horse heart)



strong interaction between histidines and divalent metal ions:
$Cu^{2+} > Ni^{2+} >> Ca^{2+}$ or Zn^{2+}

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Metal-ion coordination with histidines

-Used for protein separation and purification:

recombinant proteins with “His” tags

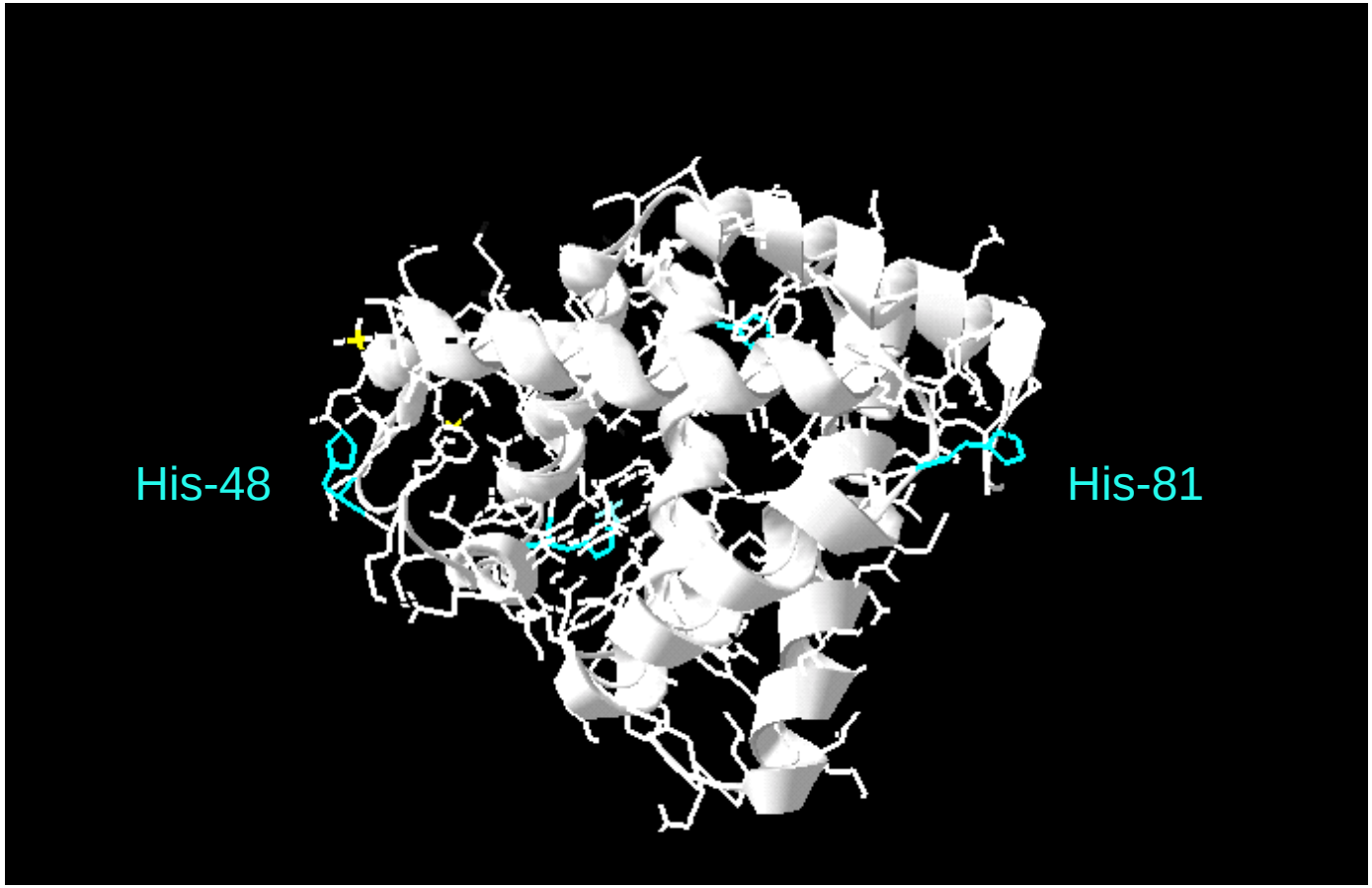
naturally occurring proteins with surface-exposed histidines can act as contaminants on chromatographic columns of this type

in some cases the goal is to purify naturally occurring proteins with surface-exposed histidines

-A general method for creating biofunctionalized surfaces

Fundamental understanding of adsorption process needed to:
control orientation, tune energetics (selectivity), avoid denaturation

Structure of myoglobin



Dimensions [Å]:
44 x 44 x 20

orientation of adsorbed protein depends upon the distribution of histidines

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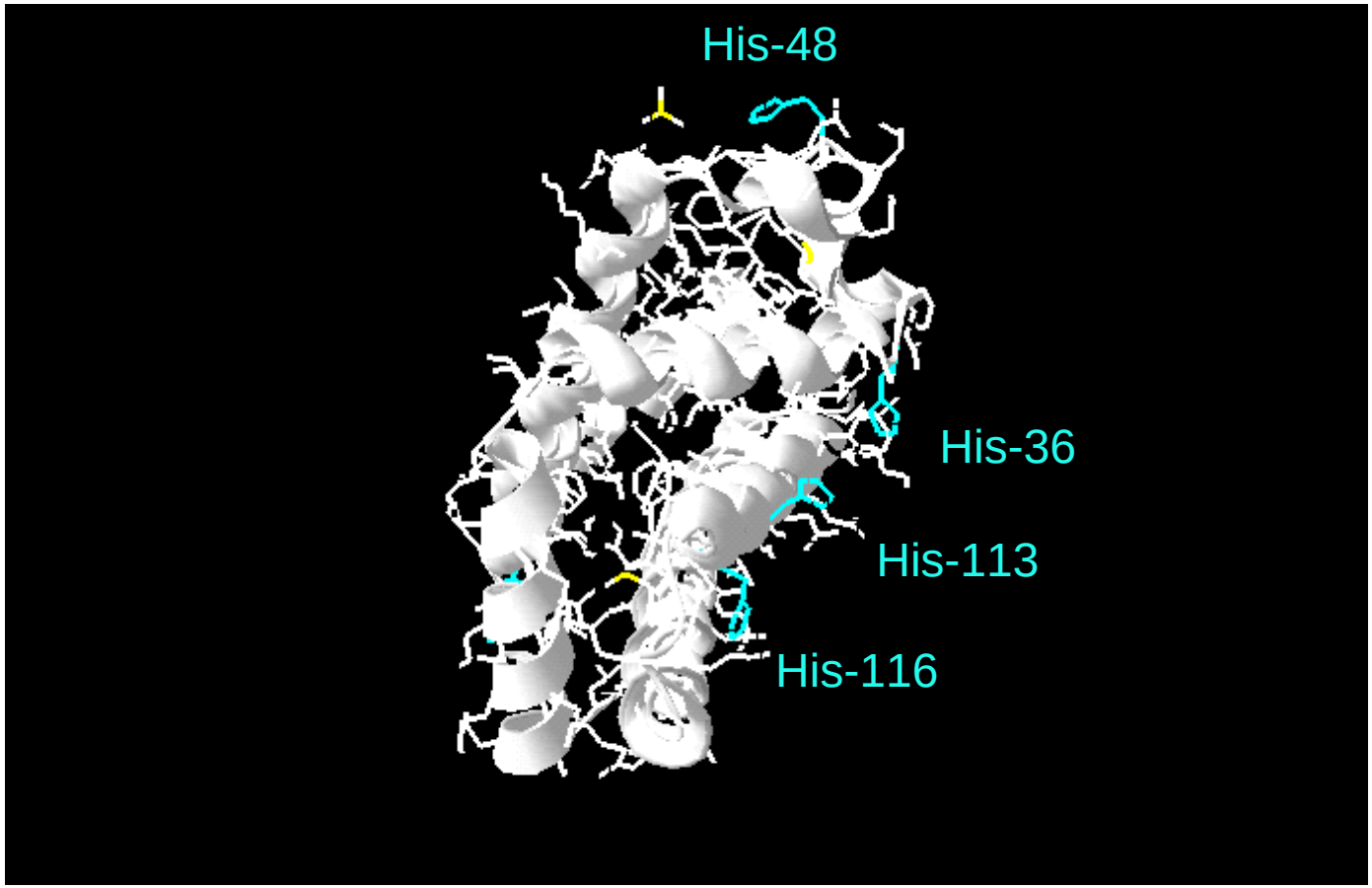
Structure of myoglobin



orientation of adsorbed protein depends upon the distribution of histidines

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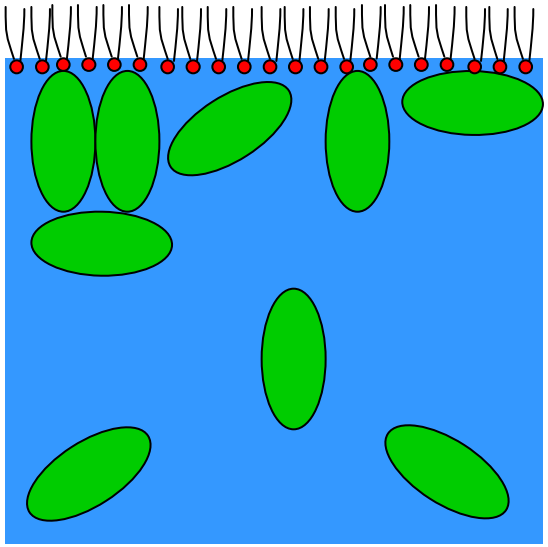
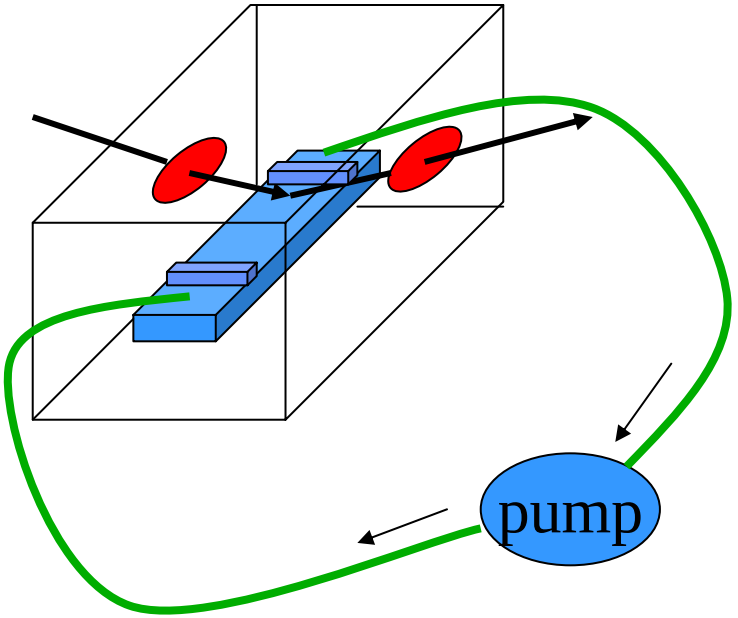
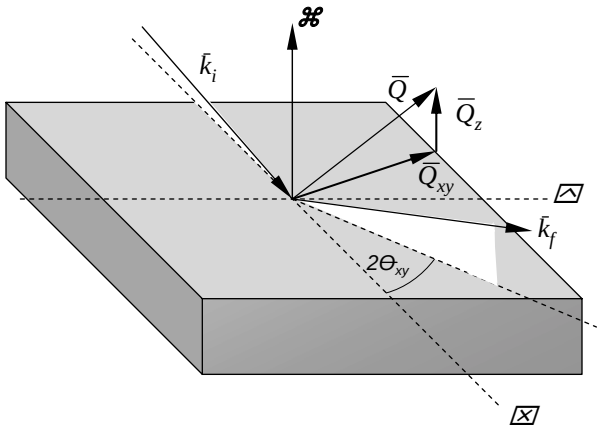
Experimental set-up





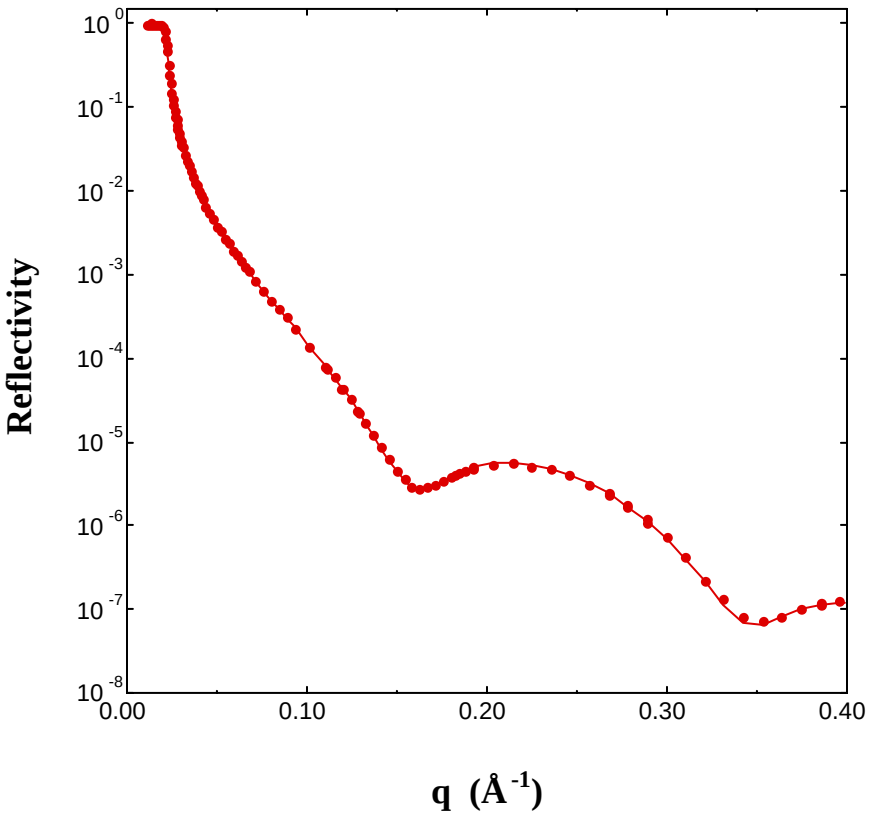
circulate metal ions and myoglobin into the subphase underneath the lipid layer

X-ray and neutron grazing incidence scattering techniques



neutrons: reflection only
X-rays: reflection and diffraction

Fix q_z here for grazing incidence diffraction (GID)



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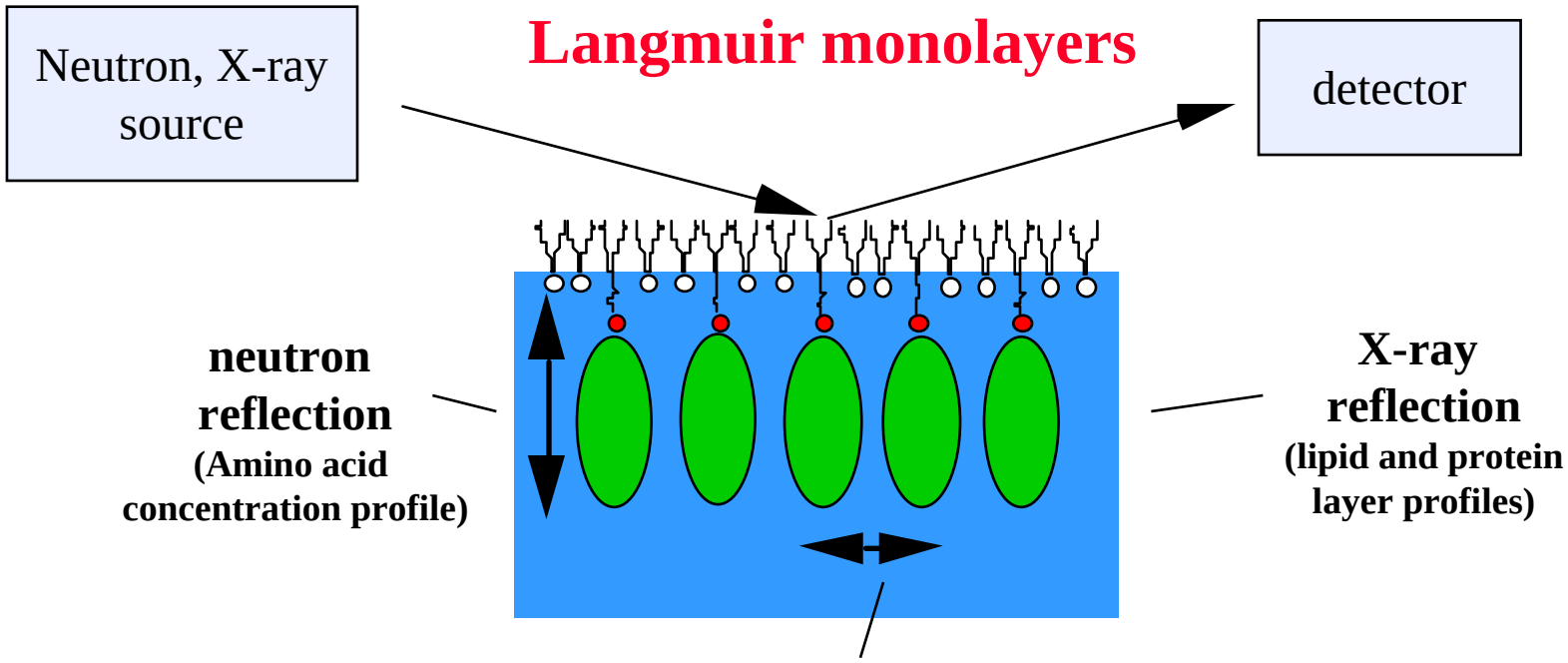
X-ray and neutron grazing incidence scattering techniques



Results

a. Protein conformation / orientation (NR, XR)
   -final state
   -evolution of layer structure with time

b. Evidence for two stages (GIXD, NR, XR)
   -stage 1: reversible
   -stage 2: irreversible

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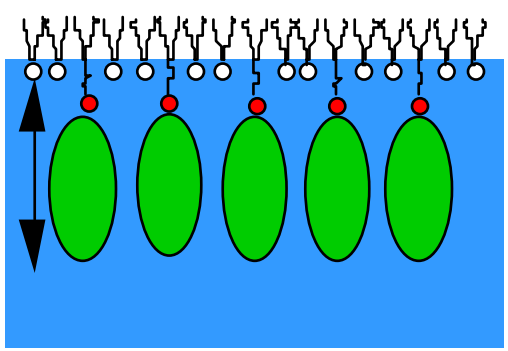
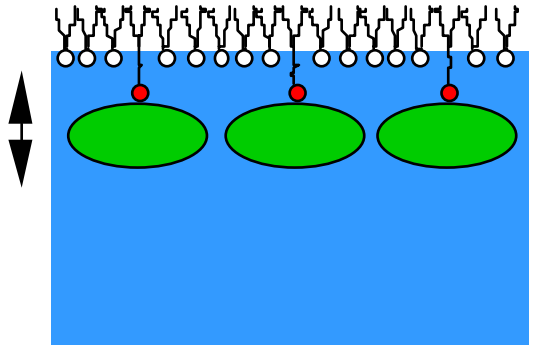
Results: A. protein conformation

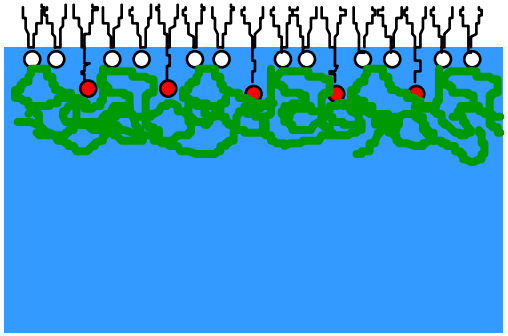
neutron (and X-ray) reflection
probes amino acid segment profile



end-on



side-on



denaturation

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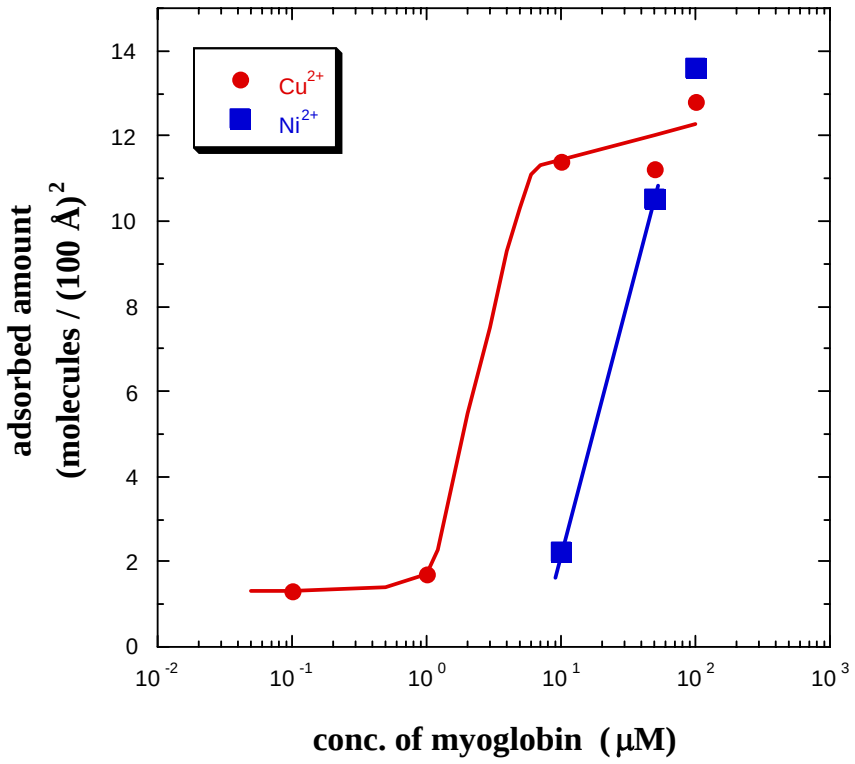
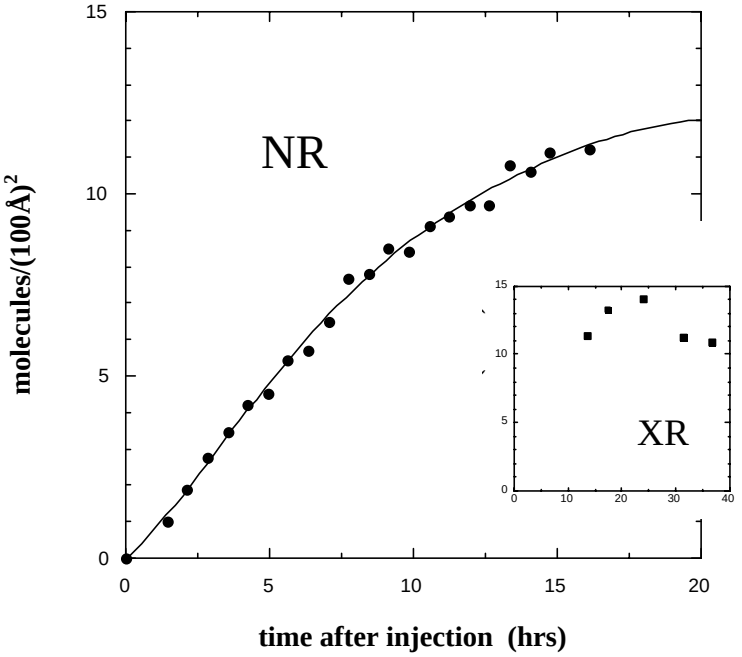
Results - surface pressure

"final" state



time dependence



Slow kinetics allow study of evolution of protein layer during the adsorption process!

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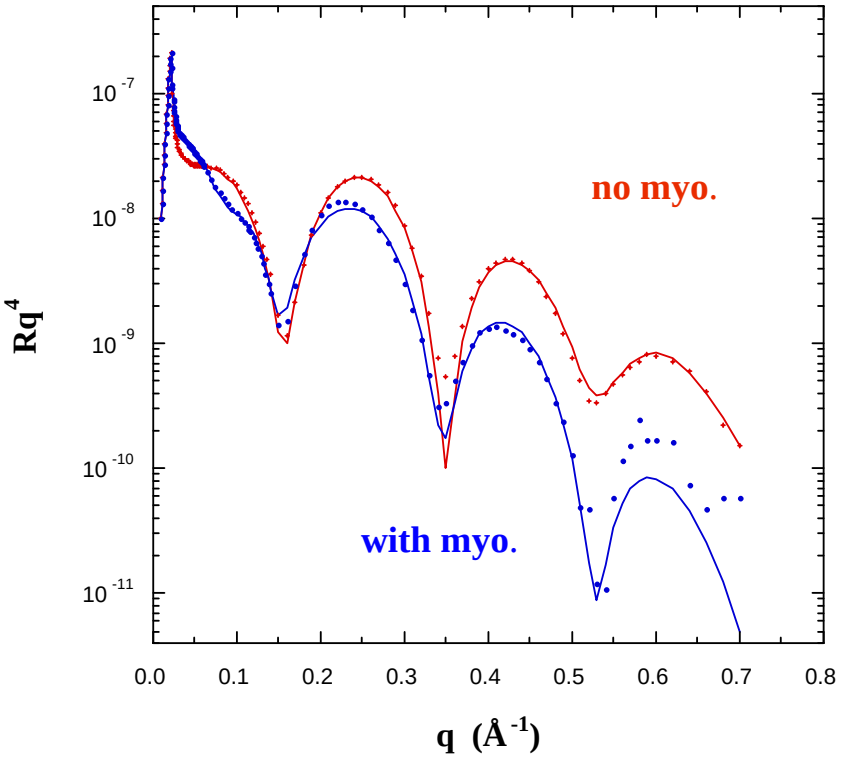
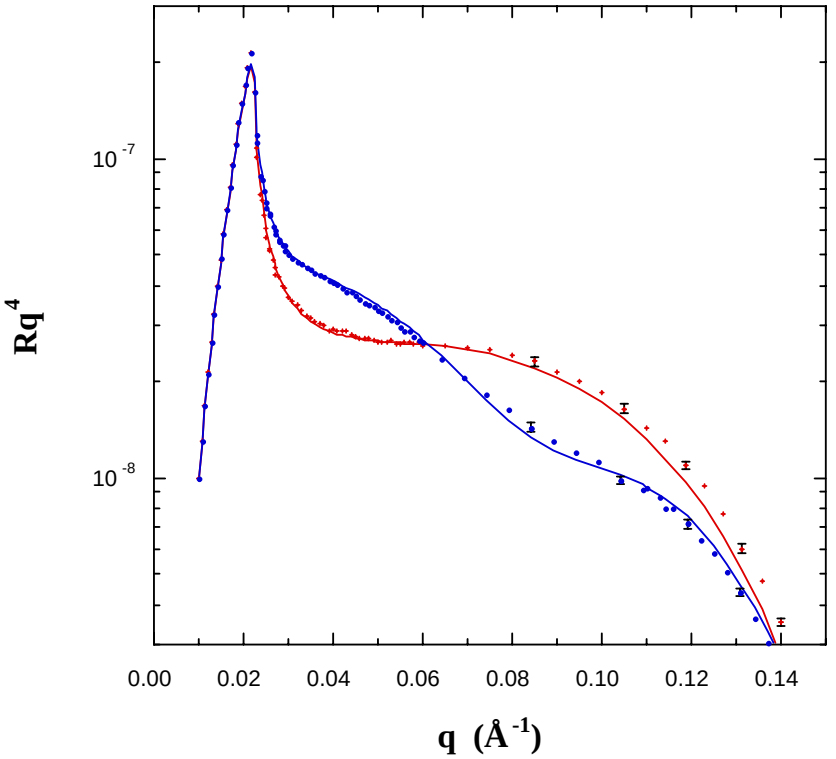
Results - X-ray reflection

chelated Cu^{2+} ions, 10 μM myo.





In-house X-ray source is sufficient to get the dimension of the protein layer in the final state!

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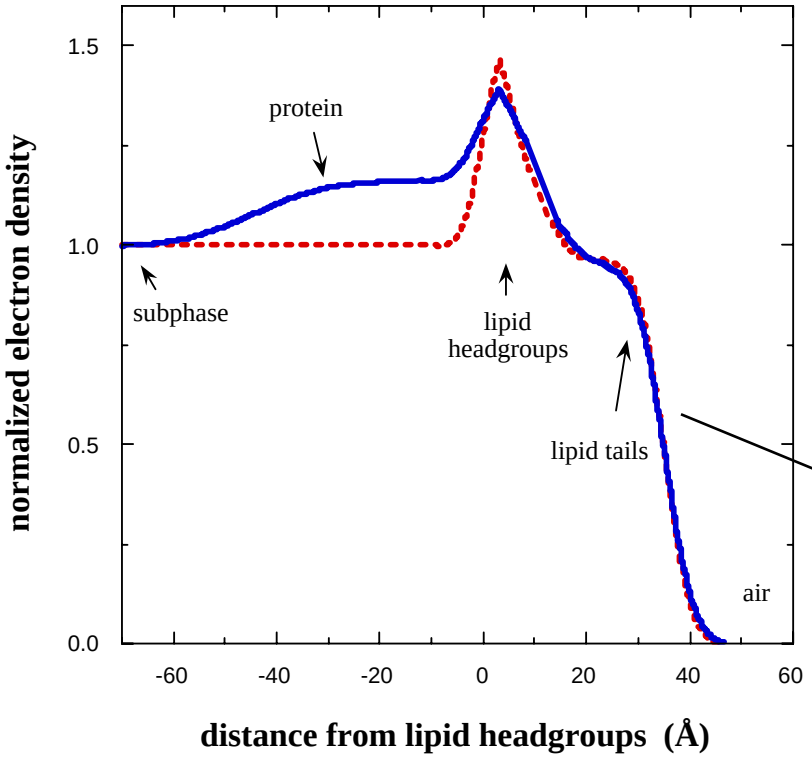
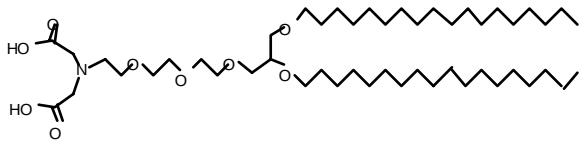
Results - X-ray reflection





DSIDA

tail layer constrained
by area/molecule
and # of electrons

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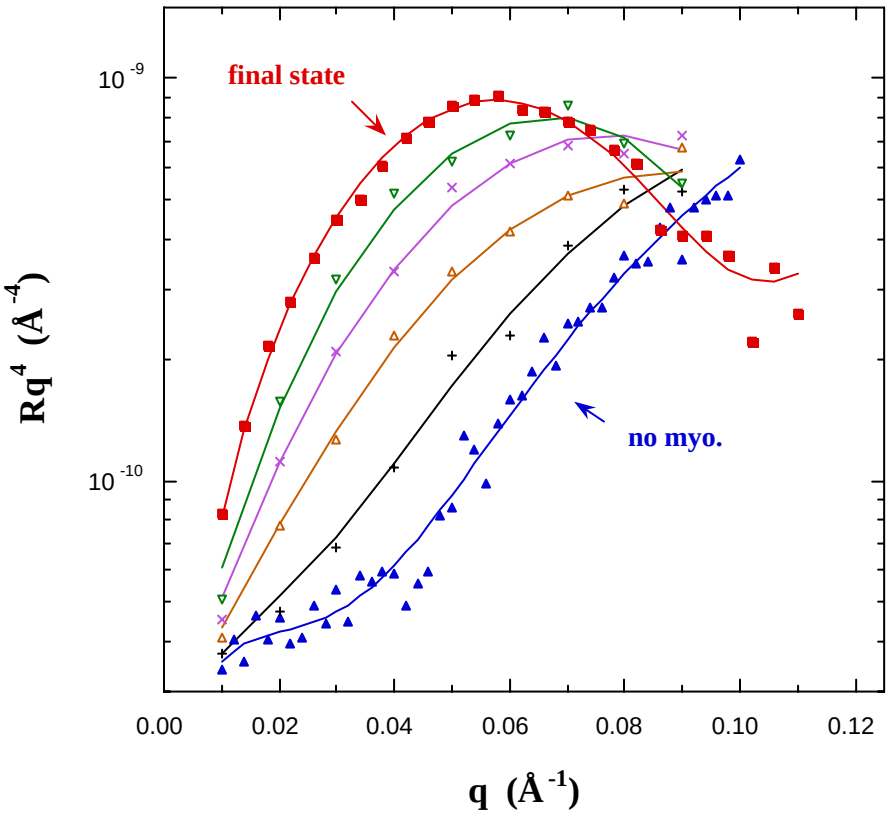
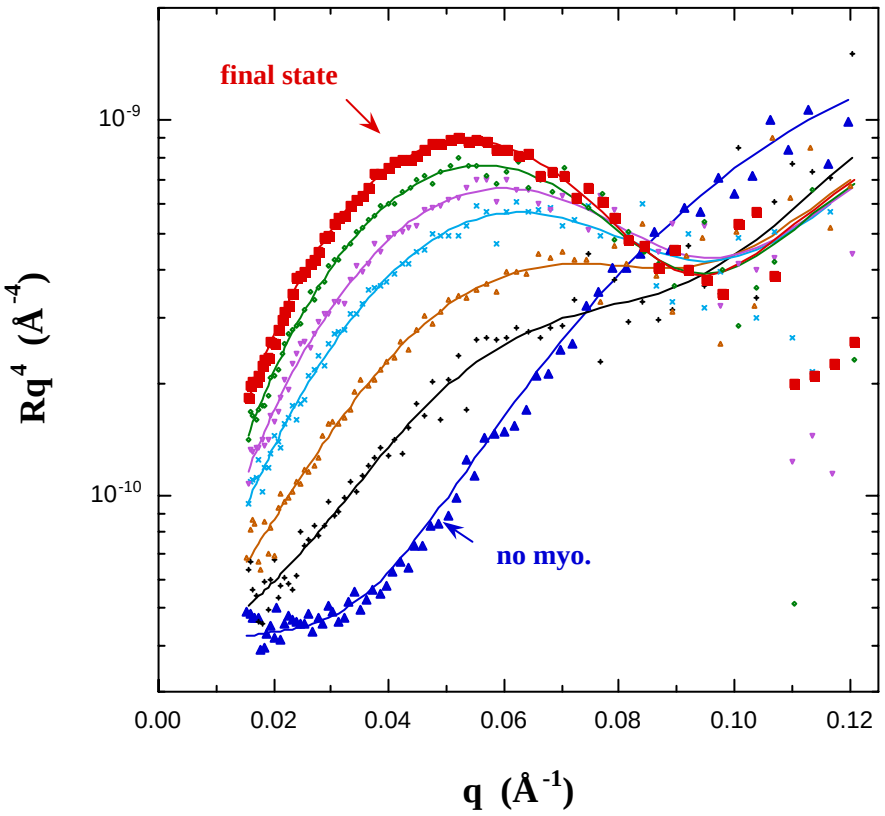
Neutron reflection, H_2O (time dependence)

Cu^{2+} ions



Ni^{2+} ions



Each curve obtained in roughly 40 minutes

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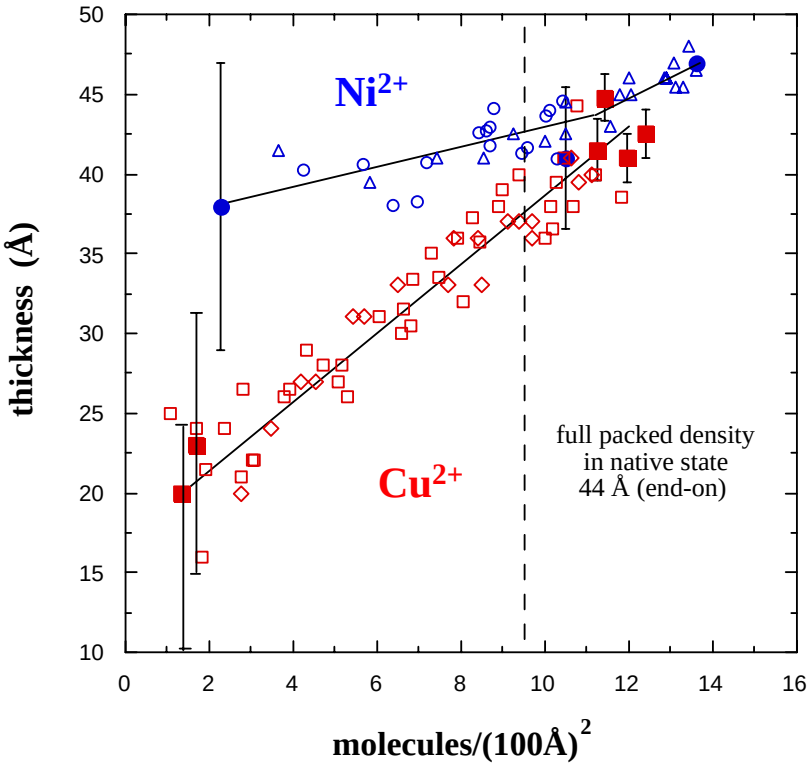
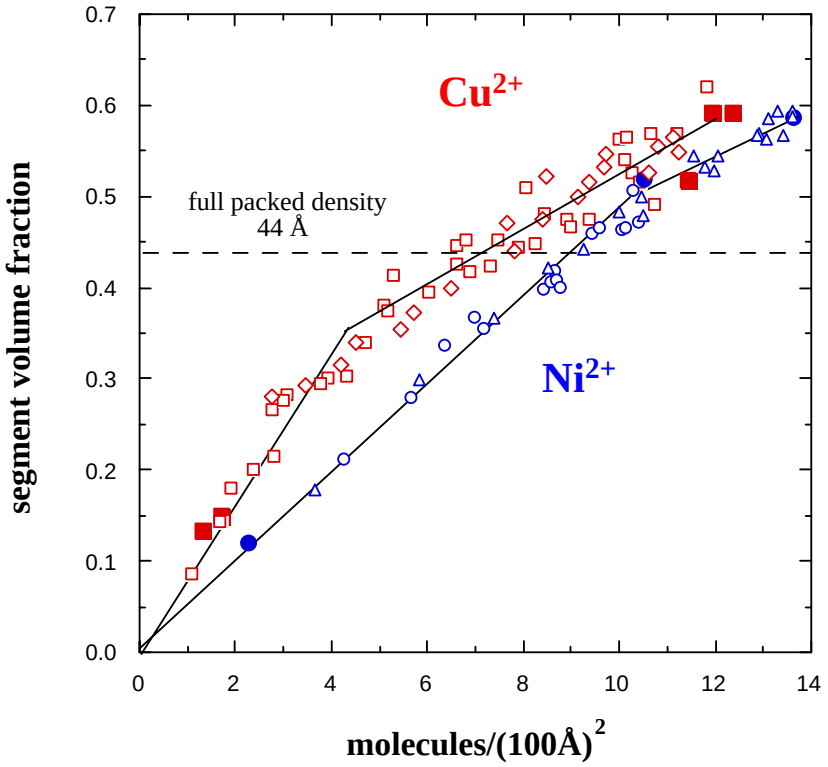
Summary

myoglobin dimensions [Å]: *44 x 44 x 25*





Isolated chains adsorb in a much thinner layer with Cu^{2+} than with Ni^{2+}

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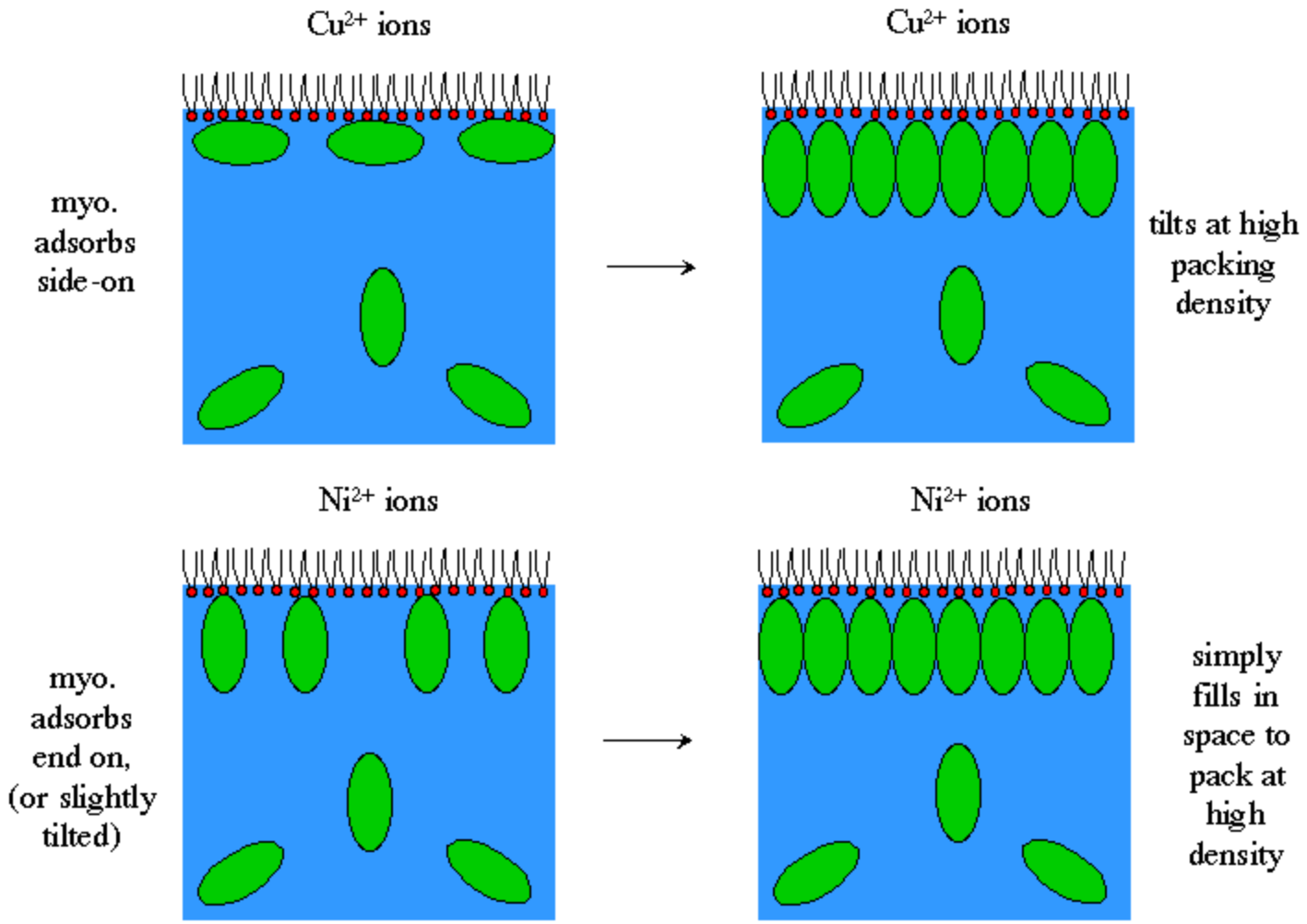
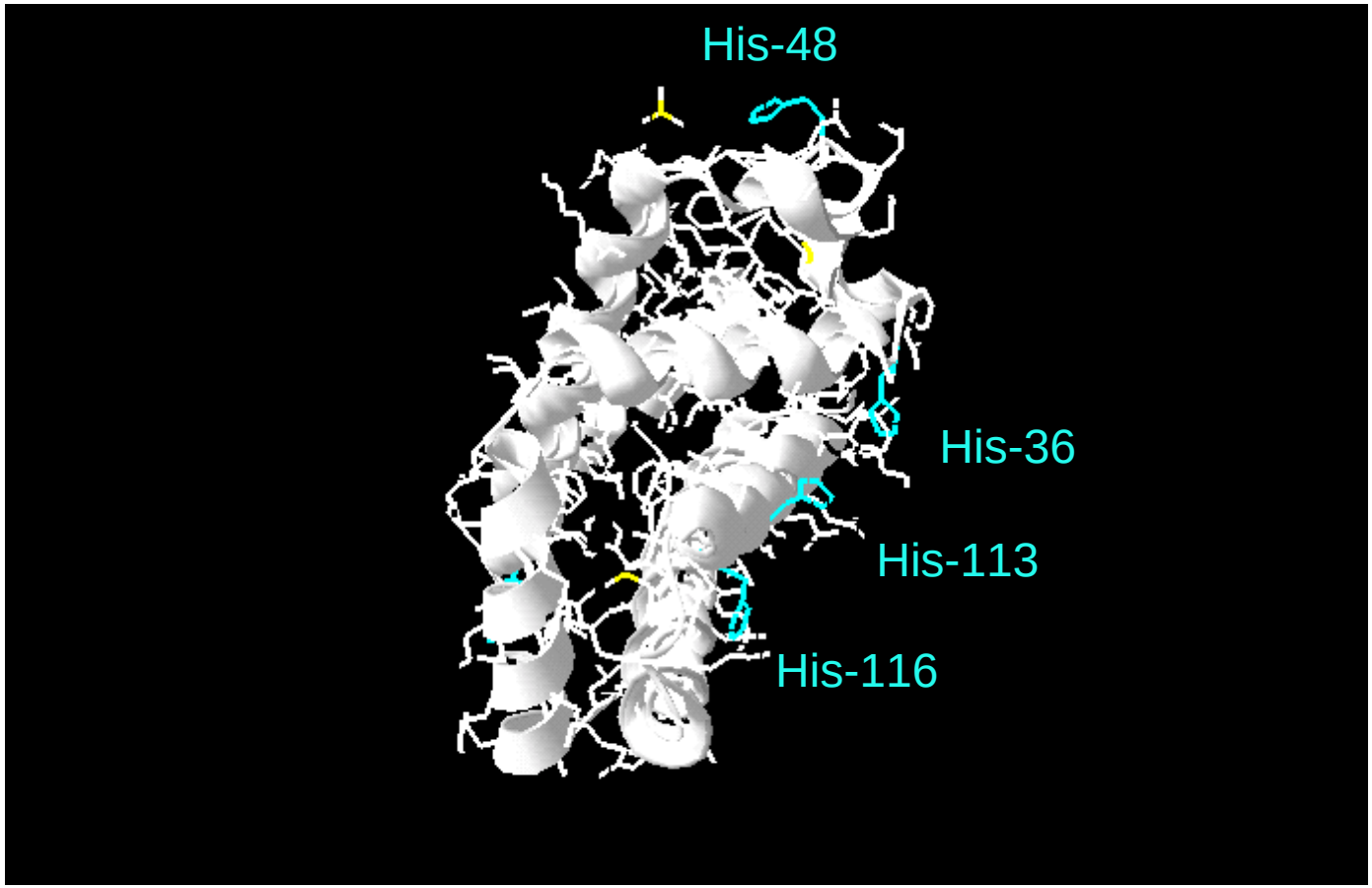
Possible interpretation



Possible interpretation



Binding by multiple histidines in case of Cu^{2+} , but His-48 or His-81 for Ni^{2+}

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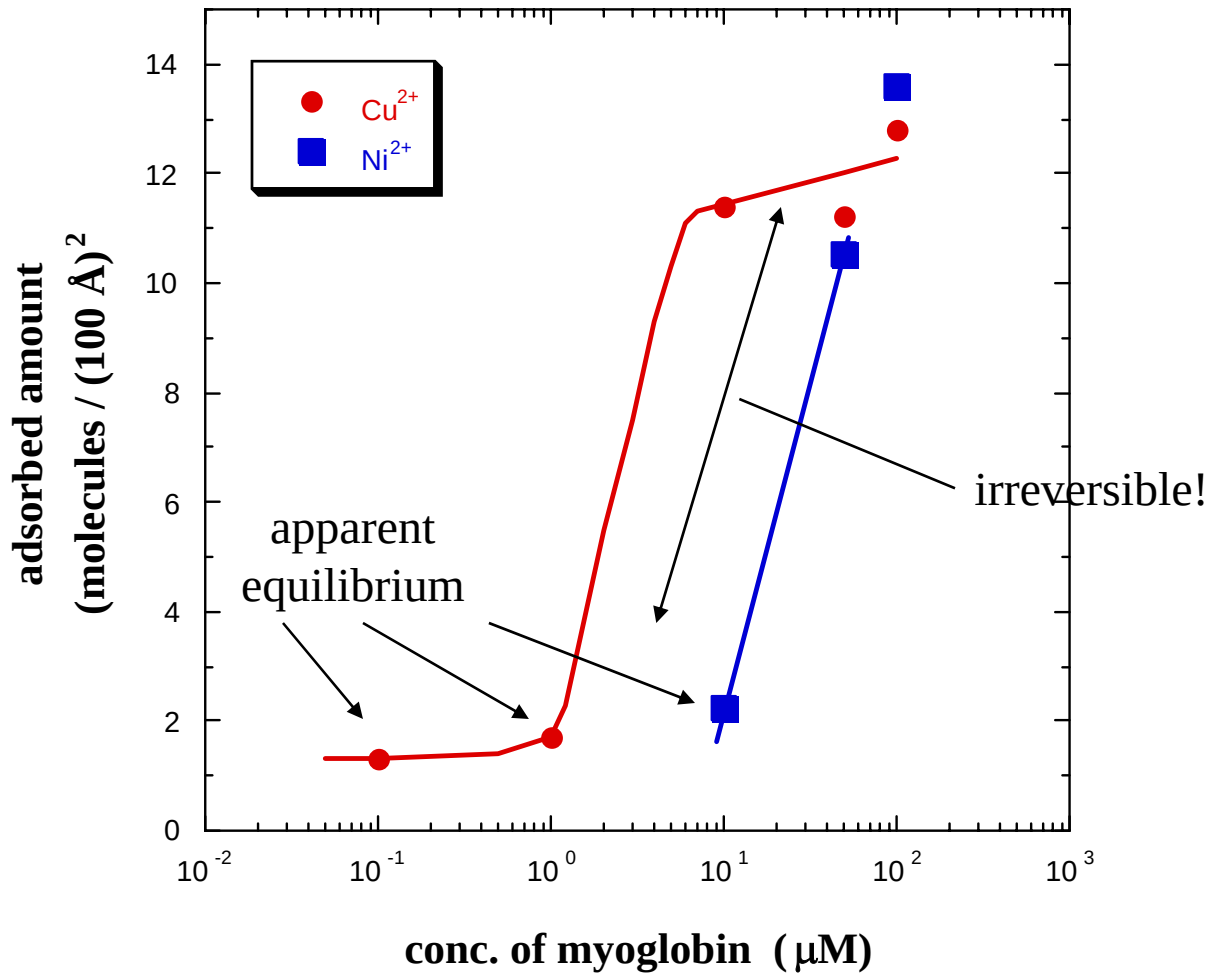
Results: B. Evidence for two stages

Evidence #1. "final" state



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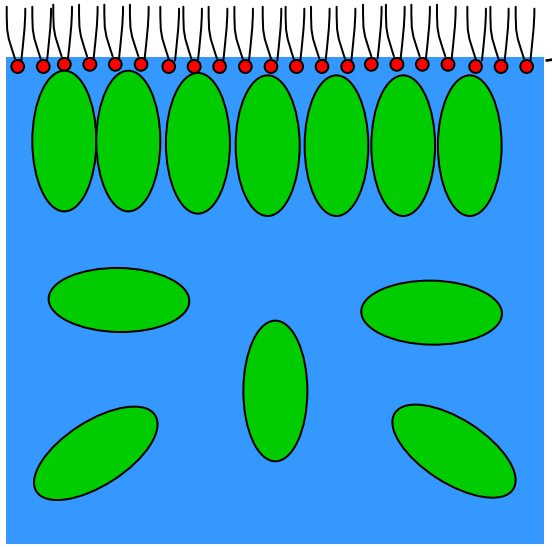
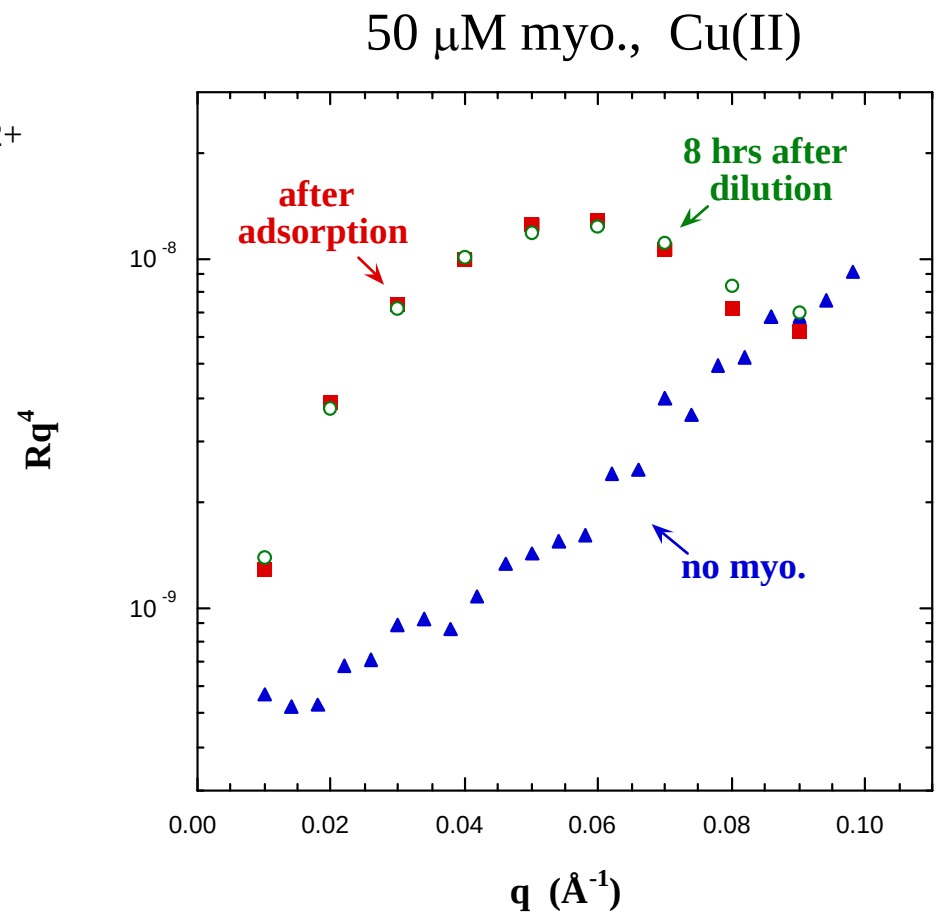
Subphase exchange shows irreversibility



diluted from
50 μM to 1.4 μM



Irreversible in fully packed state!

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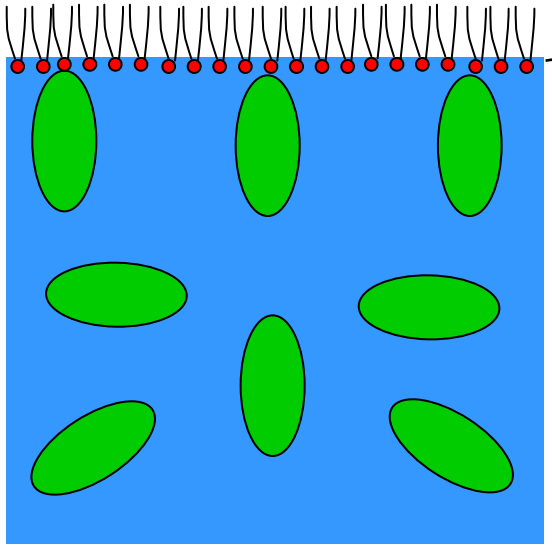
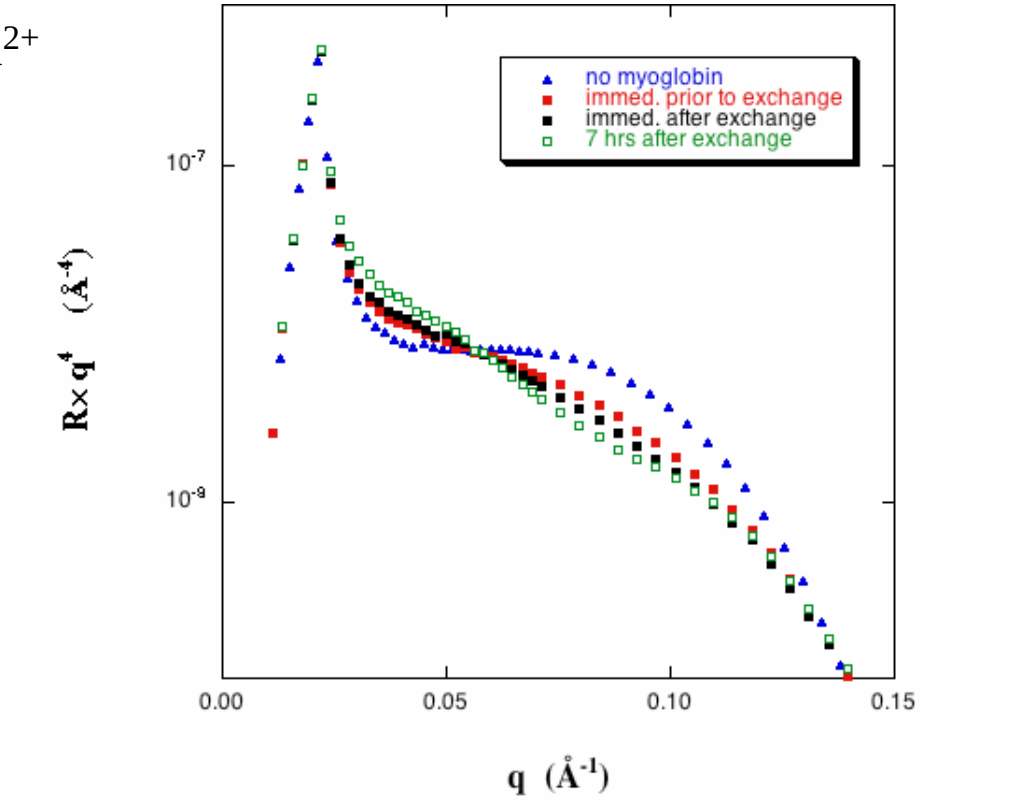
Subphase exchange shows irreversibility



diluted from
60 μM to 10 μM

60 μM myo, Ni^{2+}



Irreversible even at moderate coverage with Ni^{2+}

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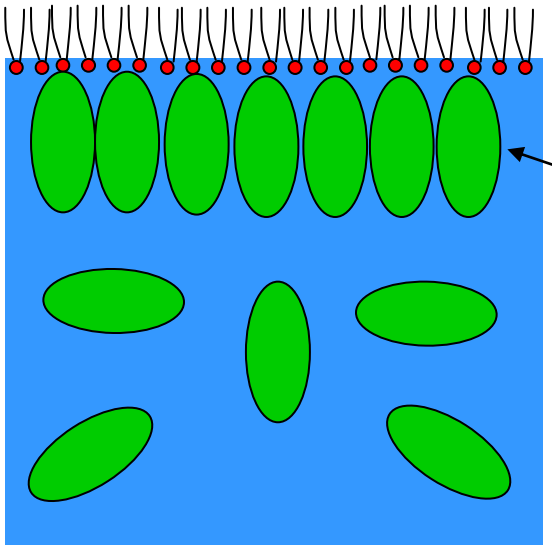
Results: B. Evidence for two stages

Evidence #2. Different time scales for disruption of lipid packing structure and accumulation of adsorbed protein



grazing incidence X-ray diffraction
(2-D crystal structure of lipid tails)

neutron and X-ray reflection
(amino acid concentration profile)

constant pressure - 40 mN/m

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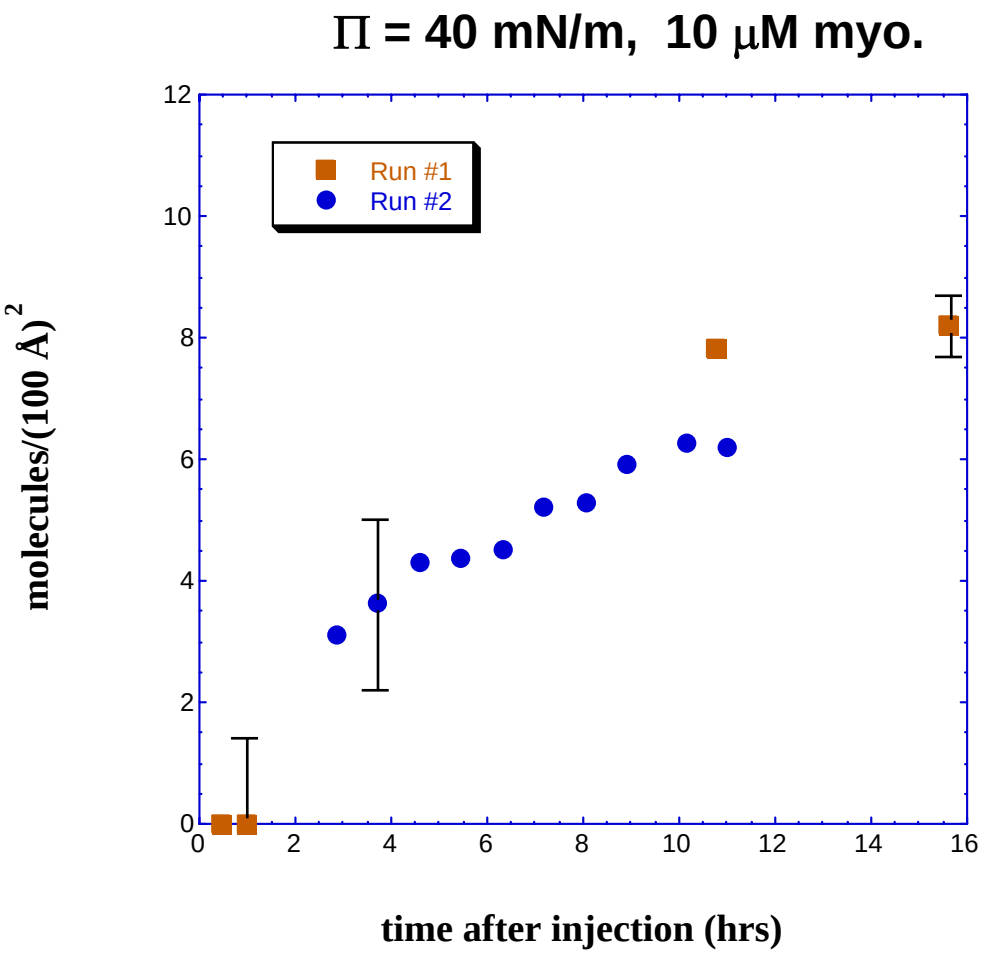
Constant pressure



2 hrs after injection very little protein has adsorbed!

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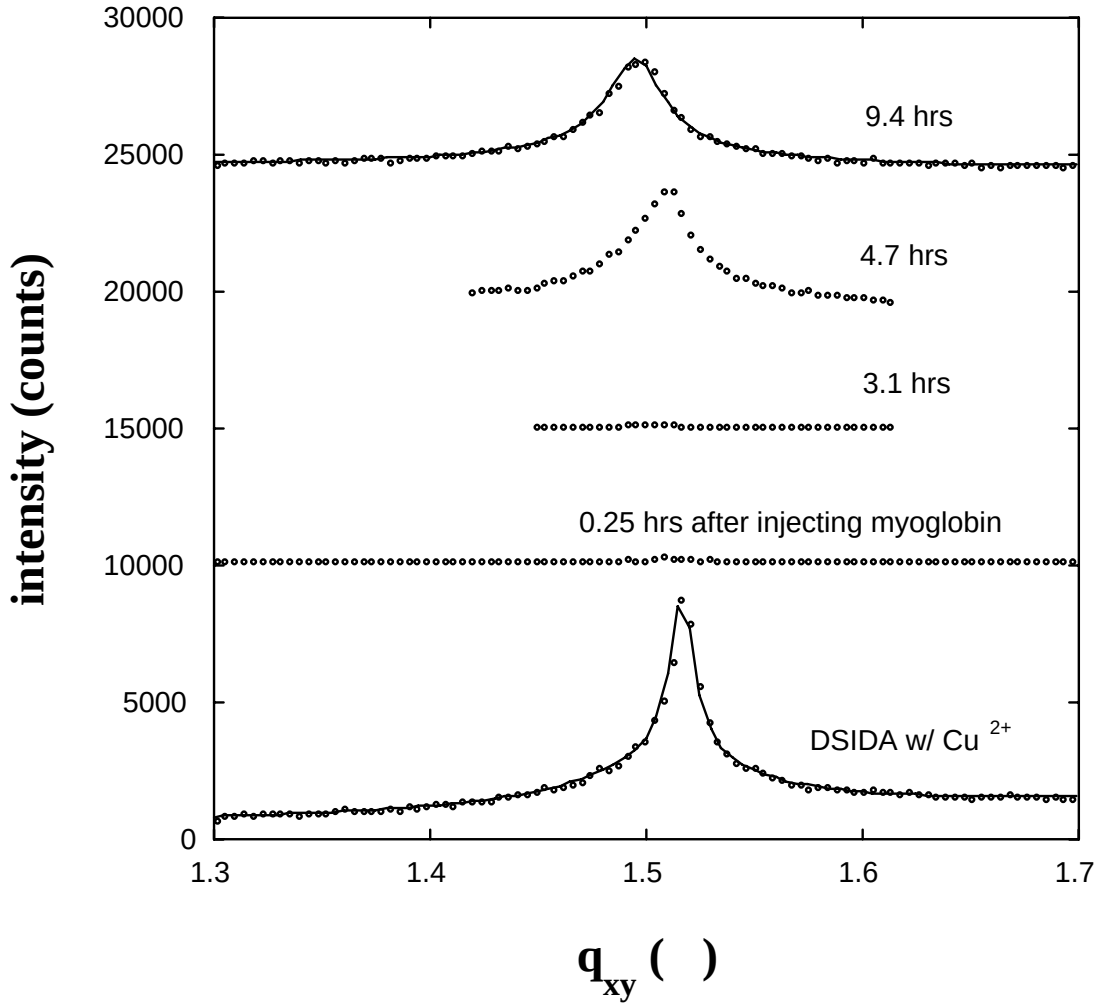
Cu^{2+}, 40 mN/m - Bragg Peak



At 0.25 hr after injection, crystallinity is no longer detected in the film!

Crystalline packing returns at ~ 5 hrs after injection

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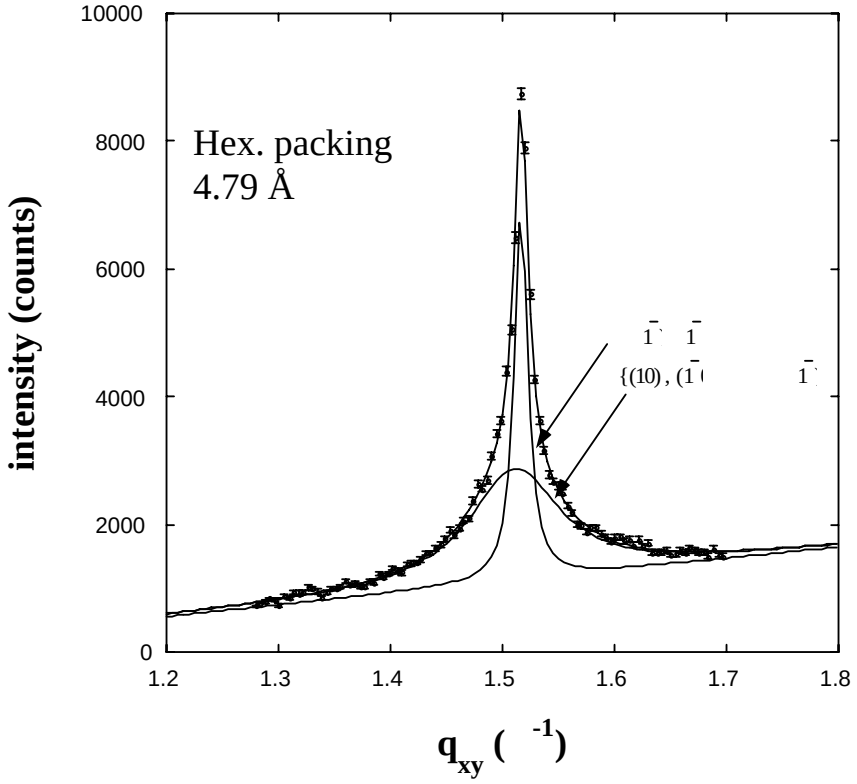
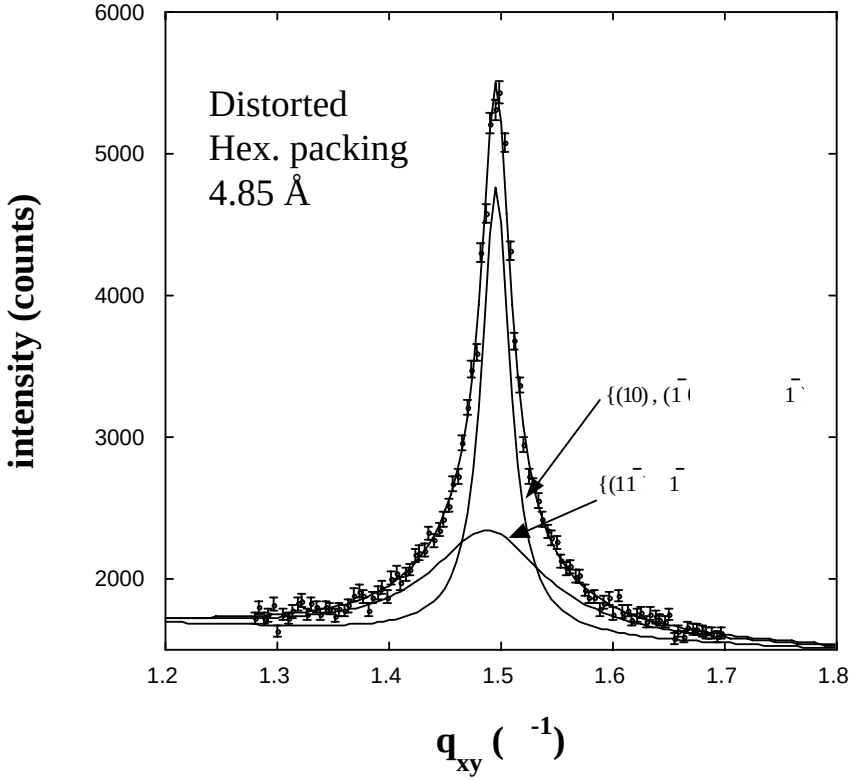
Constant pressure - Bragg Peak

Before adding myoglobin



9.4 hrs after adding myoglobin



Diffraction peak returns: broader and at lower q

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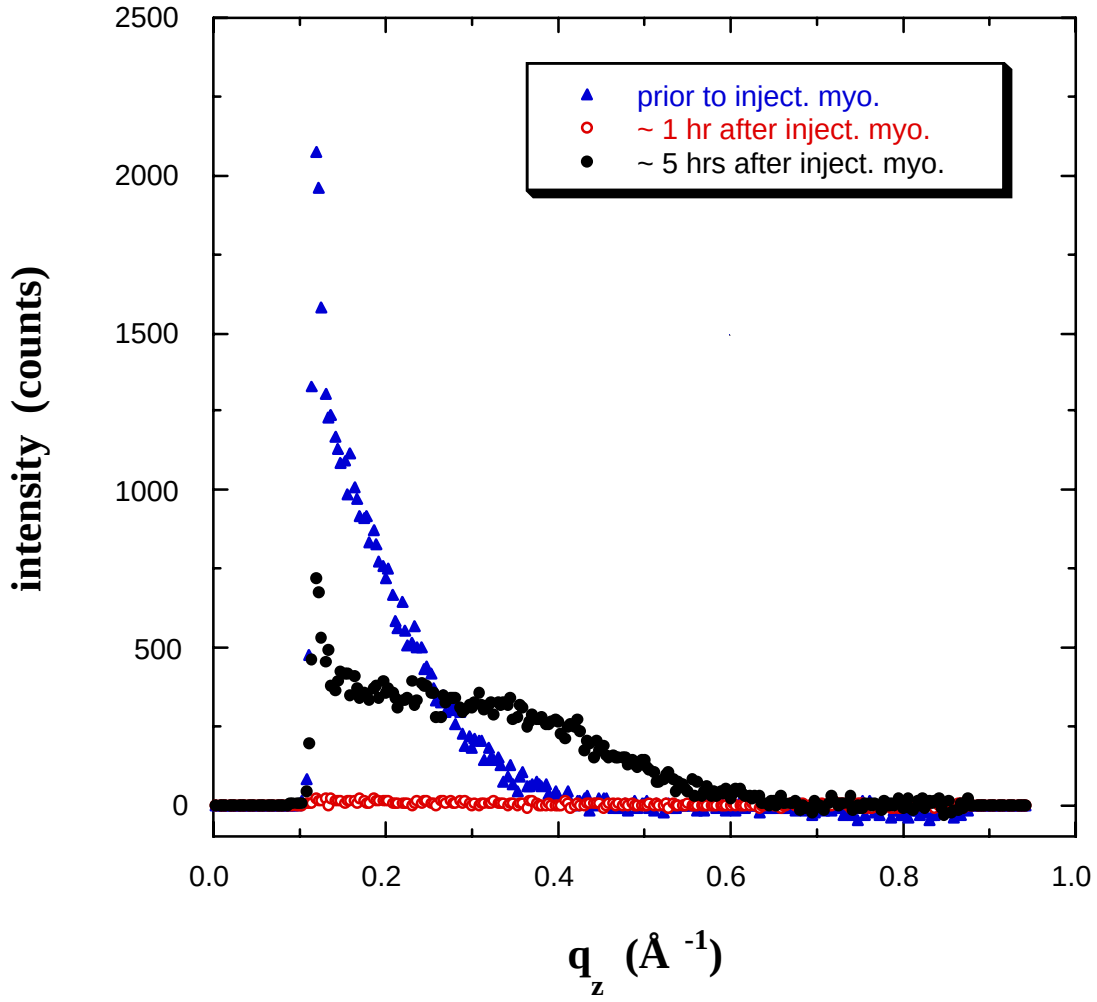
Constant pressure - Bragg Rod



Prior to injection, tails are vertical, crystalline packing

At 1 hr after injection, no crystallinity is detected in the film!

Crystalline packing returns at ~ 5 hrs after injection - tails are tilted

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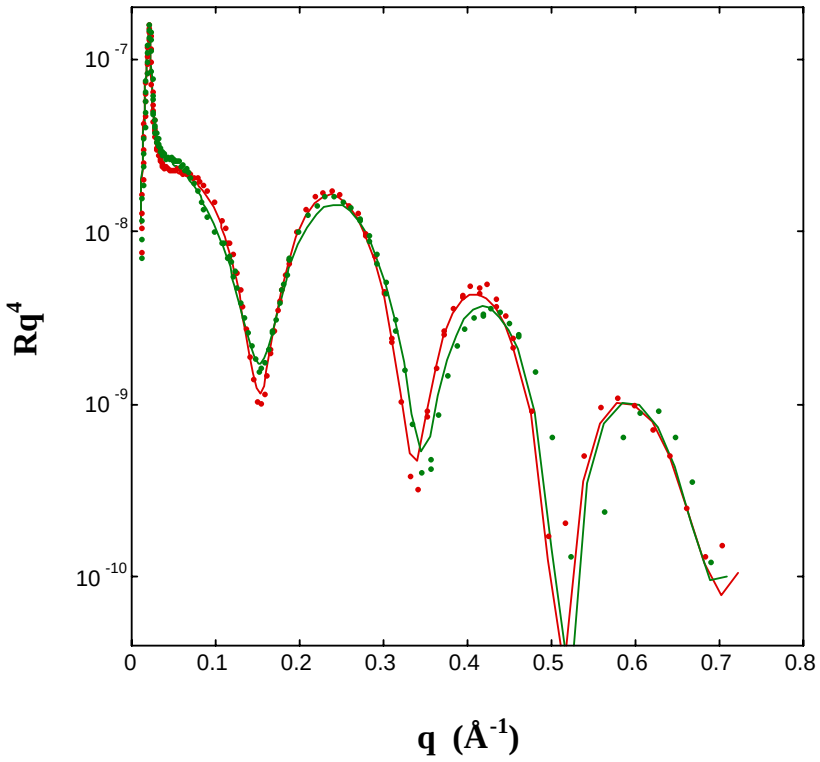
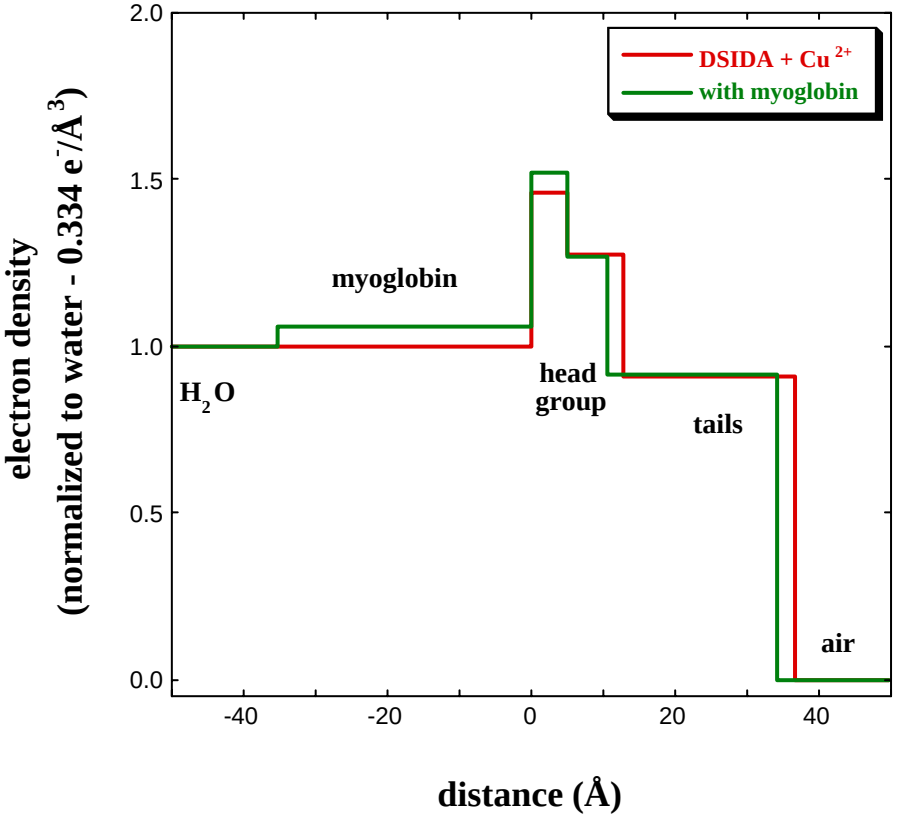
Constant pressure

0.9 hrs after injecting myoglobin





very little adsorbed protein: thick. and vol. fract. uncertain

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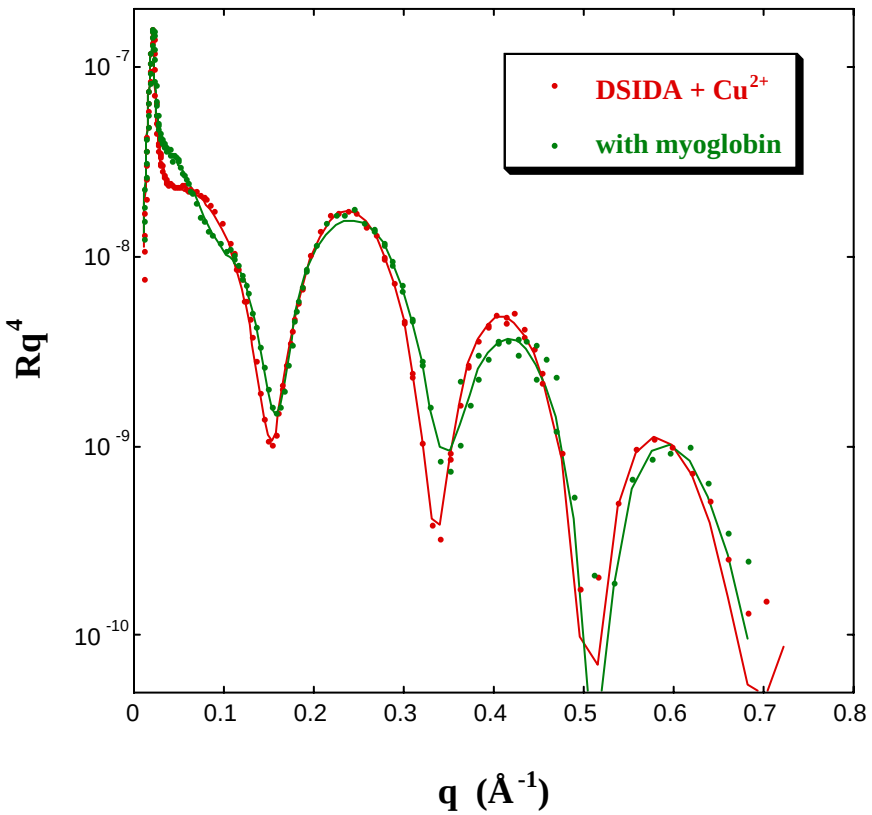
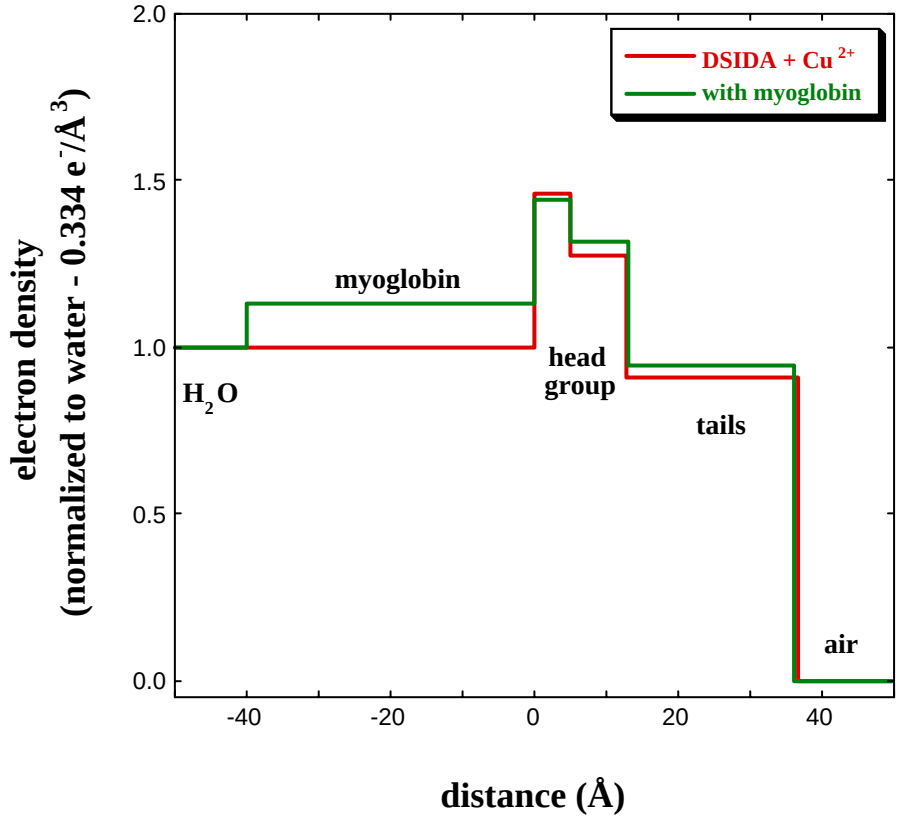
Constant pressure

3 .7 hrs after injecting myoglobin





protein layer can be observed! thickness = 40 Å, vol. fract. = 0.31

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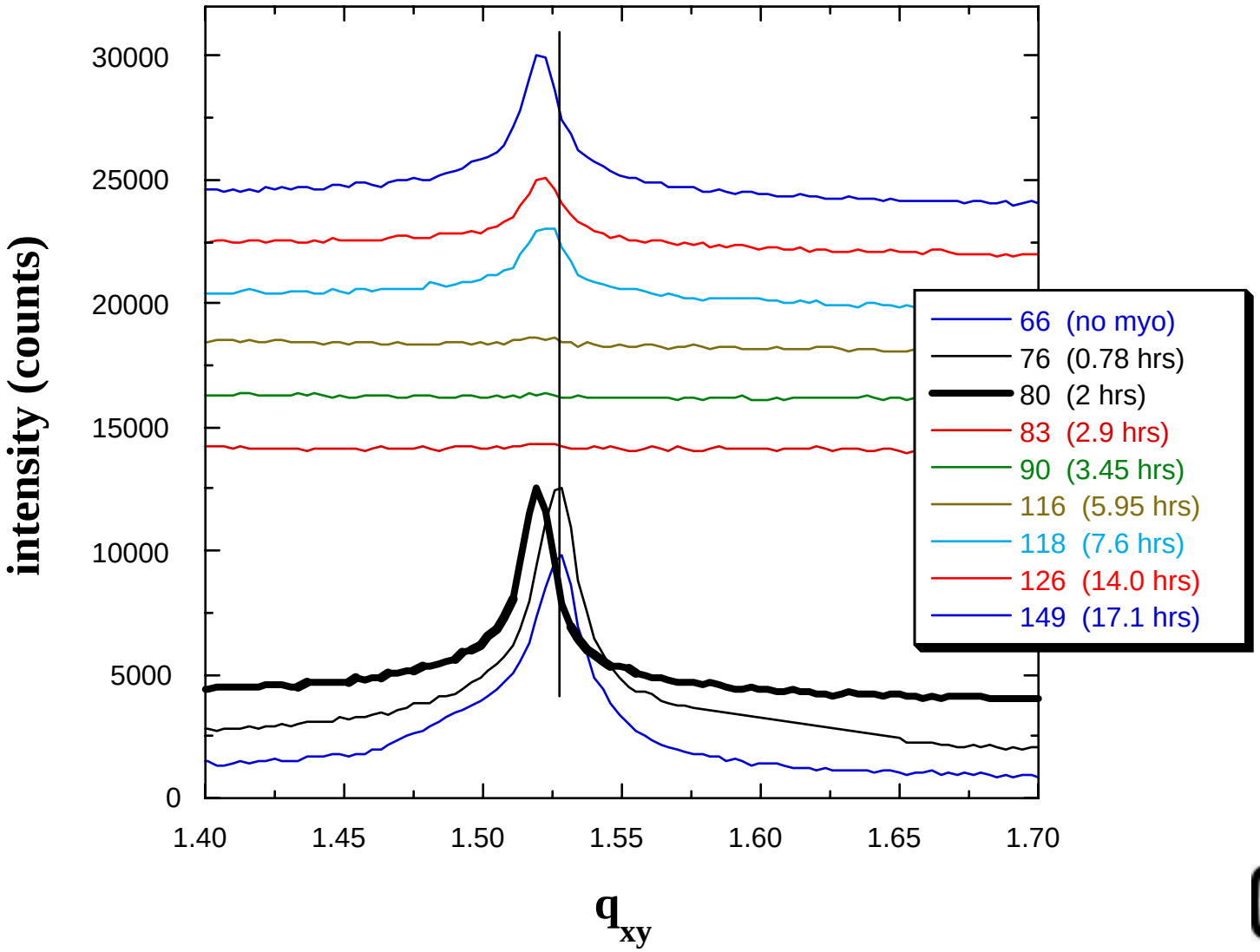
Ni^{2+}, 40 mN/m - Bragg Peak



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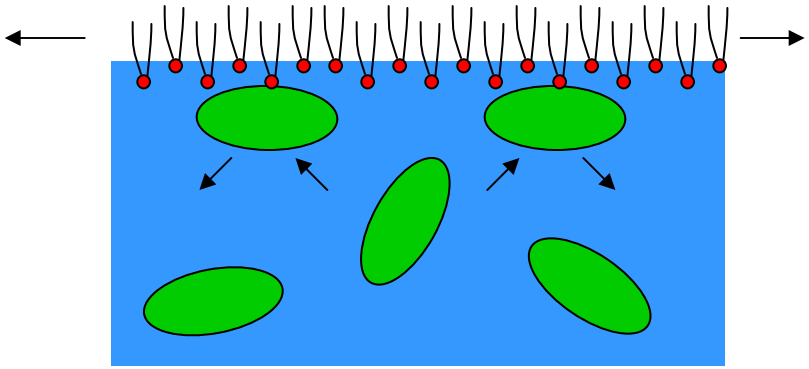
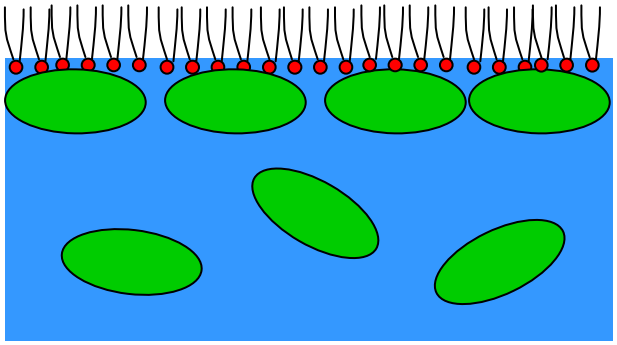
Proposed interpretation



(a)

Initial stage - reversible adsorption



(b)

2nd stage - irreversible adsorption and lipid recrystallization

Summary

Protein associations with lipid membranes:

Grazing incidence scattering techniques provide insight into:

evolution of adsorbed layer structure

protein orientation

denaturation upon adsorption

effect of protein interactions on lipid film structure

initial stage - reversible

later stage - irreversible

Acknowledgements

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APS - CMC CAT
NIST - NG7
LANSCE - SPEAR

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